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Enzyme formation and function revisited

D.S.Robertson
205, Pickersleigh Road, Malvern, Worcestershire, WR14 2QS, (ENGLAND)
Tel : 01144- 01684- 575637
E-mail : kao34@dial.pipex.com
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ABSTRACT

It is shown that the observations made of enzyme operation can be unified by considering that these compounds operate in conjunction with polyphosphoric acids which are incorporated into the enzyme structure during formation. Examples of the function of enzymes in hydration-dehydrating, deamination and decarboxylation reactions are presented. Physiological enzyme reactions such as pepsinogen and trypsin in the human digestive system and other reactions in metabolism are described. Enzyme inhibition is also discussed.

KEYWORDS

Enzyme formation;
Structure;
Operation;
Inhibition.

INTRODUCTION

An extensive literature exists regarding the operation of enzymes and present hypotheses concerning this function include the existence active centres within an enzyme which are stereochemically matched to the reacting chemicals, the formation of a complex compounds which disintegrate to give the observed products along the unchanged enzyme, and alteration of the three dimensional form of an enzyme (conformation) to become compatible with different reacting chemicals[1-3]. The principle reactions in biosystems are hydration and dehydration in the forming and breaking of peptide and glycosidic bonds, dehydration in the formation of esters, reduction, oxidation, the release of carbon dioxide from the carboxylic acid group (decarboxylation) and the removal and decomposition of amino groups (deamination). The spatial arrangement of groups in organic compounds are also involved in these reactions. A large number of number of enzymes have been identified and the three dimensional structure defined. No two enzymes have exactly the same structure and from the limited number of biochemical reaction types many structurally different enzymes must be involved in the same reactions. It follows that these must contain active centres with identical stereochemical and stereophysical characteristics. In enzyme reactions experimental plots of the change in concentration of the reaction product with time using a given amount of reactant (substrate) and enzyme has three sections, a transient section where the amount of reaction product produced in a given period of time increases non linearly, a linear section where the amount of reaction product produced in a given period rises linearly and finally a section in which the amount of reaction product produced in a given period remains constant. No single mathematical equation derived from chemical kinetics is known which fits the entire extent of this relationship. An alternative concept of enzyme operation is that an enzyme is a protein which during formation stage becomes associated with an active chemical agent which is involved in enzyme reactions and which is continu-

ously renewed unlike the case for normal chemical reactions where the concentration of all of the reactants diminishes continuously. The work below describes this concept.

The formation of enzymes

Enzymes are proposed as consisting of the combination of a protein formed by dehydration of amino acids by polyphosphoric acid and incorporating active units based on the various spatial forms of polyphosphoric acid or monophosphoric acid. Phosphate ion is the dominant inorganic ion in metabolic cells (TABLE 1). The next most dominant ion is potassium and from the concentration values given in TABLE 1 0.15 mole of potassium ion requires 0.05 mole of phosphate ion to form potassium orthophosphate K3(PO4). The concentration of phosphate ion is in excess of this requirement. This means that some phosphate ion exists in cells as alternative forms. It has been shown that in mixtures of water and phosphorus pentoxide the various forms of polyphosphoric acid are dominant when the molecular ratio of these two compounds is one to one[4] In cells the free water content is low. These conditions will therefore encourage the existence of polyphosphoric acids in intracellular fluids. In support of this conclusion it is known that potassium dihydrogen phosphate

TABLE 1: Ion concentration in and metabolic fluids[13]

Intercellular			
Cations	**Mol/L**	**Anions**	**Mol/L**
Na+	0.14	Cl-	0.105
K+	0.004	HCO_3-	0.05
Mg++	0.004	PO_4--	0.006
Ca++	0.01	SO_4--	0.002
Organic			
Cations		**Mol/L**	
Acid		0.006	
Protein		0.012	
Intracellular			
Cations	**Mol/L**	**Anions**	**Mol/L**
Na+	0.01	Cl-	0.002
K+	0.15	HCO_3-	0.008
Mg++	0.05	PO_4--	0.285
Ca++	0.006	SO_4-	0.05
Organic			
Cations		**Mol/L**	
Acid		0.008	
Protein		0.055	

More than one value of intracellular phosphate ion concentration is given in the literature. Values from 0.008 to 0.020 Mol/L and 0.10 Mol/L are recorded[14,15]

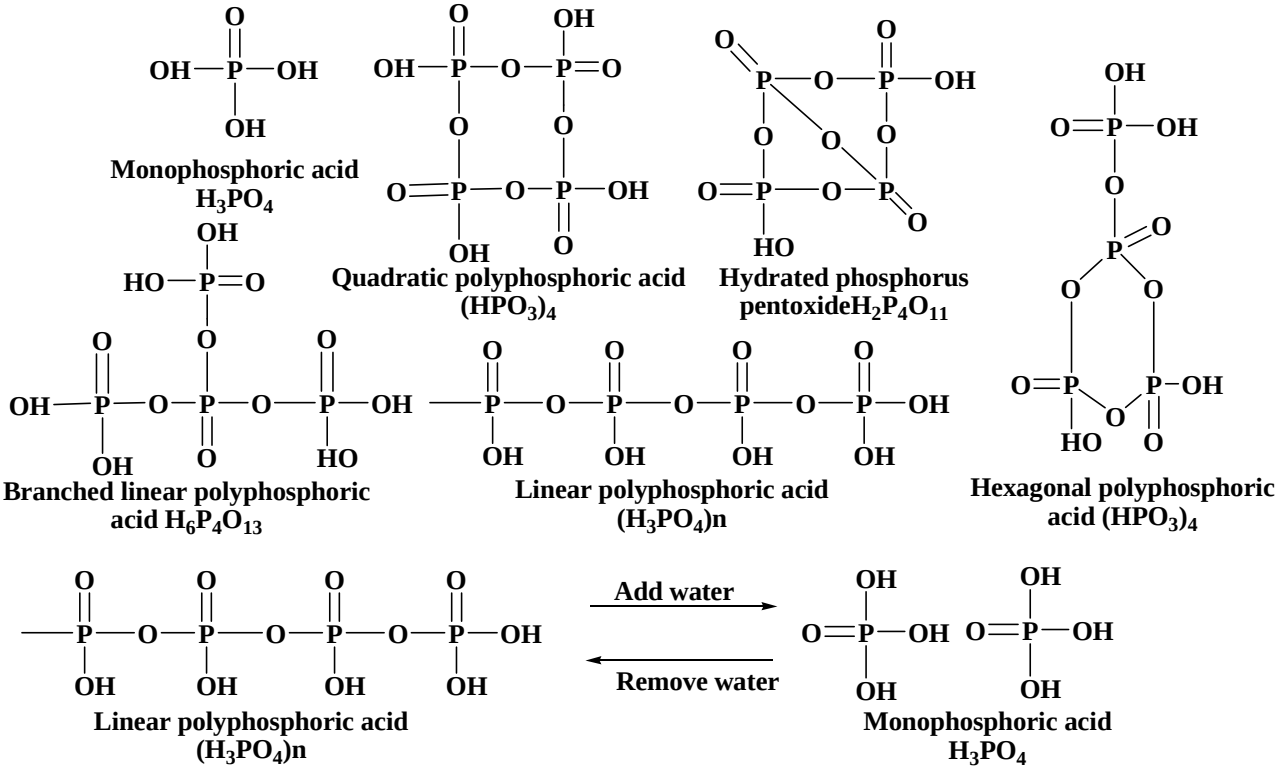

Figure 1: The forms of polyphosphoric acid

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(KH_2PO_4) and dipotassium hydrogen phosphate (K_2HPO_4) possess a strong tendency to form polyforms and polyphosphates have been identified in cells[5]. Adenine triphosphate is found in all cells and is a salt of polyphosphoric acid.

Polyphosphoric acid exists in various spatial forms as shown in figure 1 and undergoes reversible hydration to monophosphoric acid. Polydiphosphoric acid has four replaceable protons with the values $pK_1 = 1.7$, $pK_2 = 1.95$, $pK_3 = 5.98$ and $pK_4 = 8.74$. Monophos phoric acid has three replaceable protons and the values of the constants are $pK_1 = 2.12$, $pK_2 = 7.21$ and $pK_3 = 12.3$[4]. Comparison of the same constants for each acid shows that when the monophosphoric acid groups are formed the pH of the cell increases and the reverse in the case formation of polyphosphoric acid groups. The pH value of the isoelectric point and solubility of amino acids vary with pH[6,7]. The linkage of a particular amino acid to the forming enzyme structure by dehydration as shown in figure 2 will be dependent on both these properties. Under conditions where there is no change of pH of the intracellular fluid and a sufficient concentration of molecules of the same amino acid are available these will continue to link. When a sufficient change in pH value occurs this prevents the same amino acid joining. The next amino acid with compatible characteristics joins and releases another monophosphoric acid molecule. Again a change occurs in pH with the same result of defining the characteristics of the next amino acid to join. The pH value,

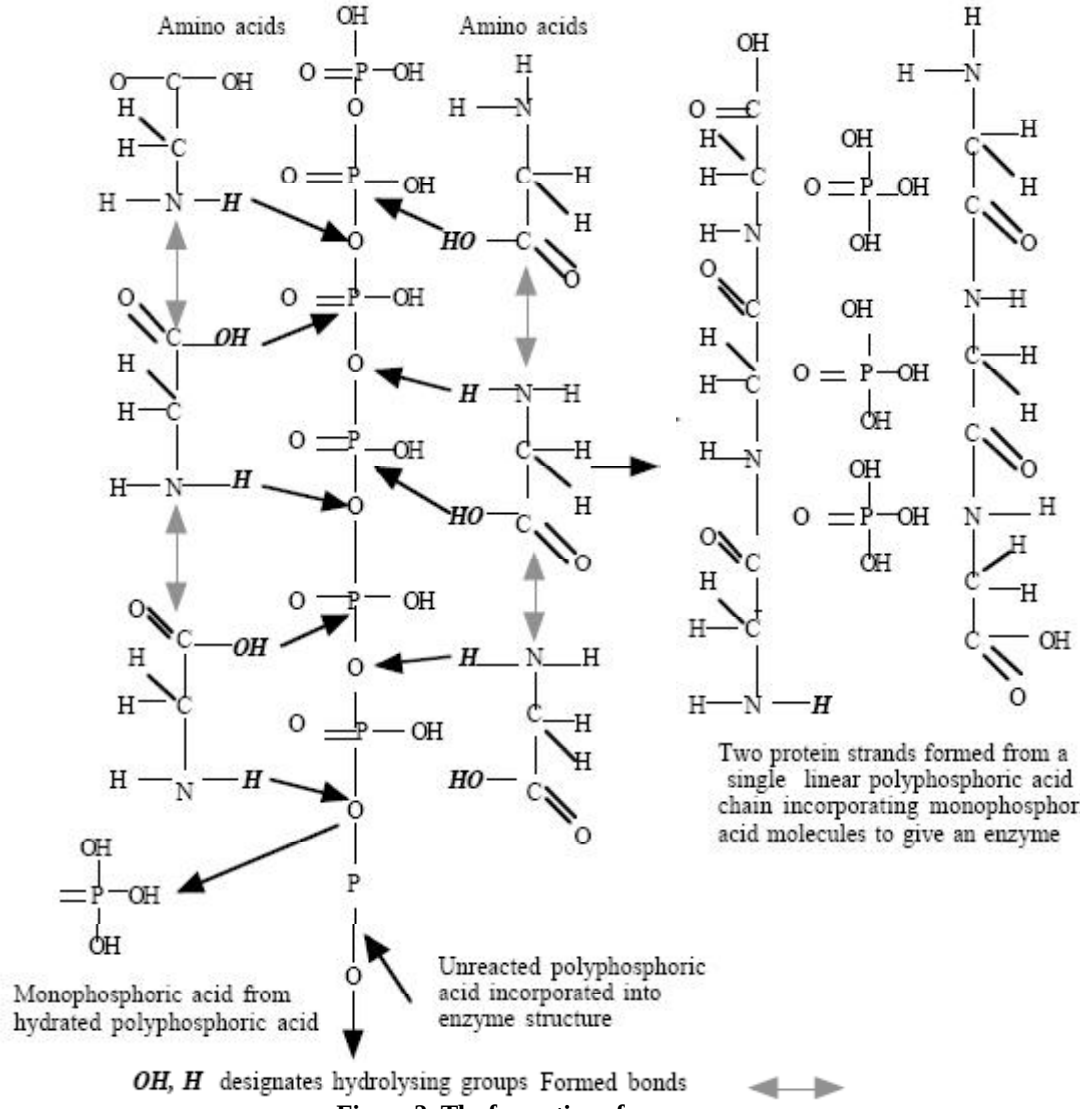

Figure 2: The formation of an enzyme

the temporal changes in this value and the spatial concentration and ionisation characteristics of the various amino acids present will determine the amino acid sequence in the enzyme produced. The physical and chemical properties of polyphosphoric acids support the formation of enzymes involving the involvement of these acids as a molecular template. For example the minimum stable length of the polyphosphoric chains has been found to be 10 monophosphoric acid units linked together. Chains with more than 500 units are considered to convert to cyclic forms and long linear chains are also considered to exist in the form of spirals[4]. These properties support the formation long chain enzymes with varying three dimensional forms.

The formation of polyphosphoric acid in cells requires a mechanism for the conversion of monopho sphoric acid to polyphosphoric acid. This requires the removal of water from the former and ultimately the removal of water from a cell. Processes available in cells for this conversion are osmosis and electro-osmosis. The former process requires that the concentration of one or more ions exterior to the cell is greater than the concentration of these ions within the cell. Electro-osmosis requires that a potential difference exists across the cell membrane. Both these conditions are present in cells. The concentration of sodium ions outside the cell is very similar to the concentration of potassium ions inside the cells and there is little sodium in the cell and little potassium outside the cell. The concentration differences and electrochemical potentials of these ions (sodium,-2.711 volts, potassium, -2.924 volts.[7]) give rise to potential difference known as the membrane potential which has a value of the order of 70 mV. Water leaving the cell supports the conversion of any monophosphoric to polyphosphoric acid giving rise to a continuous process. This movement of water towards the cell membrane will give rise to a gradient in the concentration of polyphosphoric acid such that the concentration is highest at points remote from the cell membrane i.e. towards the centre of the cell. In these regions where the free water concentration is a minimum the formation of long chain, hexagonal and other forms of polyphosphoric acids will be enhanced.

Finally the nature of the cell fluids supports the proposed mechanism of enzyme formation. The presence in the intracellular, intercellular and blood fluids of low concentrations (millimoles per litre) of ionic compounds which are normally highly soluble in aqueous media (TABLE 1) is indicative of a nature different from pure water. The viscosity of the fluids is higher than water. This supports the conclusion that these fluids exist in the colloidal state. The high concentration of water in the fluids indicates that fluids are hydrophilic colloidal fluids[8]. The colloidal material in a hydrophilic colloidal fluid can also be caused to precipitate as a gelatinous solid by reducing the temperature of the former. The solids so formed can be reconverted into a hydrophilic colloidal fluid by increasing the temperature. It is known that cells can be inactivated by freezing and reactivated by return to body temperature (37° Celsius) supporting the nature of the intracellular fluid involved as a hydrophilic colloidal fluid. Proteins and polysaccharides readily form hydrophilic colloidal fluids as do the primary alkali metal salts of the bases forming DNA and RNA[9]. The enzyme forming and other cell reactions will be favoured by taking place in a hydrophilic colloidal fluid which can be considered as a semi-solid. This restricts molecular motion (translation, rotation, vibration) to a greater extent than a free liquid allowing molecular spatial position and orientation to become effective factors in cell reactions.

TABLE 2 : The properties of some enzymes

Enzyme	Optimum operational pH	Reaction
Pancreatic lypase	8	Hydrolysis of glycerol esters of long chain fatty acids (fats)
Digestive lipase	4.0 - 5.0	As above
Pepsinogen	1.5 - 1.6	Hydrolysis of proteins
Trypsin	7.8 - 8.7	As above
Urease	7	Rupture of C-N bond. products CO_2, NH_3, H_2O
Pancreatic amylase	6.7 - 7.0	Hydrolysis of carbohydrates
Catalase	7	Decomposition of hydrogen peroxide

The functioning of enzymes

On the basis of the above proposals the properties of an enzyme e.g. optimum operational pH (TABLE 2) are dependent on the protein structure along with the relative concentration of the monophosphoric and polyphosphoric acids associated with the structure. The conditions in a given fluid (pH value, temperature, na-

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ture and concentration of cations present) are the factors which decide whether monophosphoric or polyphosphoric acid is the stable form[4]. Polyphosphoric acids are less stable against hydrolysis at low pH values (acid conditions) and the stability increases at high pH values (alkaline conditions)[4]. In extreme acid conditions (pH = 1.0) little or no polyphos phoric acid is present and the reversible hydrolysis reaction does not operate. As pH is altered towards high pH values the polyphosphoric acids become more stable against hydrolysis[4] and the reversible hydrolysis reaction can operate. In extreme alkaline conditions the stability of the polyphosphoric acids is such that the reversible hydrolytic reaction is again inhibited. The observed variation of the optimum pH among enzymes is the result of the relative amounts of monophosphoric and polyphosphoric acid associated the structural differences of the enzyme protein. This means that an enzyme with an optimum operational pH at low pH values (pepsinogen, optimum operational pH =1.5-1.6) contains more molecules of monophosphoric acid per unit enzyme volume compared with polyphosphoric acid than an enzyme with an optimum operational pH at high pH values (trypsin, optimum operational pH = 7.8-8.7). Pepsinogen will tend to be a hydrating enzyme and trypsin a dehydrating enzyme. Interaction between the former type of enzyme and a normal protein will result in hydration of the latter to amino acids and the conversion of the monophosphoric acid groups associated with the enzyme to polyphosphoric acid groups. The latter type does not readily interact with a formed protein and reacts with amino acids to give rise to a protein and the formation of monophosphoric acid groups. The optimum operational pH of an enzyme is the point at which the reversible reaction is favoured by the relative concentrations of mono and polyphosphoric acids and the nature of the physical association of these acids with the matrix of the enzyme.

It follows that the same reaction can be supported by different enzymes when these are used at a pH removed from the optimum. Under these conditions the rate of the reaction will depend on the relative concentrations of monophosphoric and polyphosphoric acid associated with the enzyme structure i.e. trypsin will act as a hydrating enzyme at pH = 1.5 - 1.6 although not as efficiently as pepsinogen at the same pH. This means that the rate of a particular enzyme reaction is dependent on enzyme type and concentration and is demon-

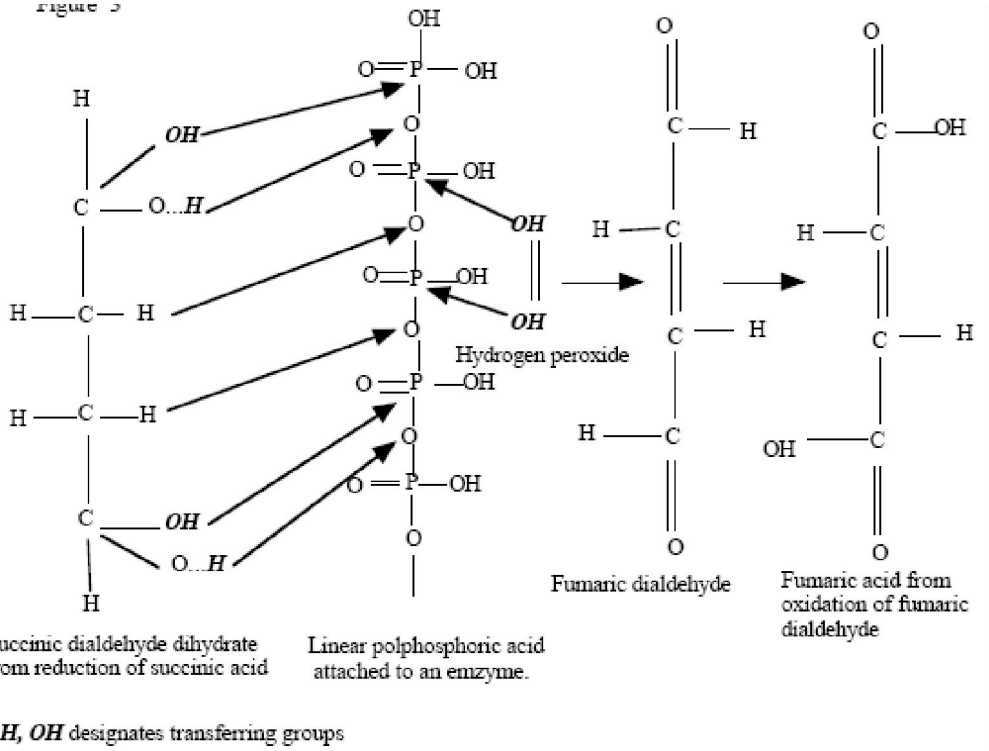

Figure 3: The enzyme oxidation of succinic acid

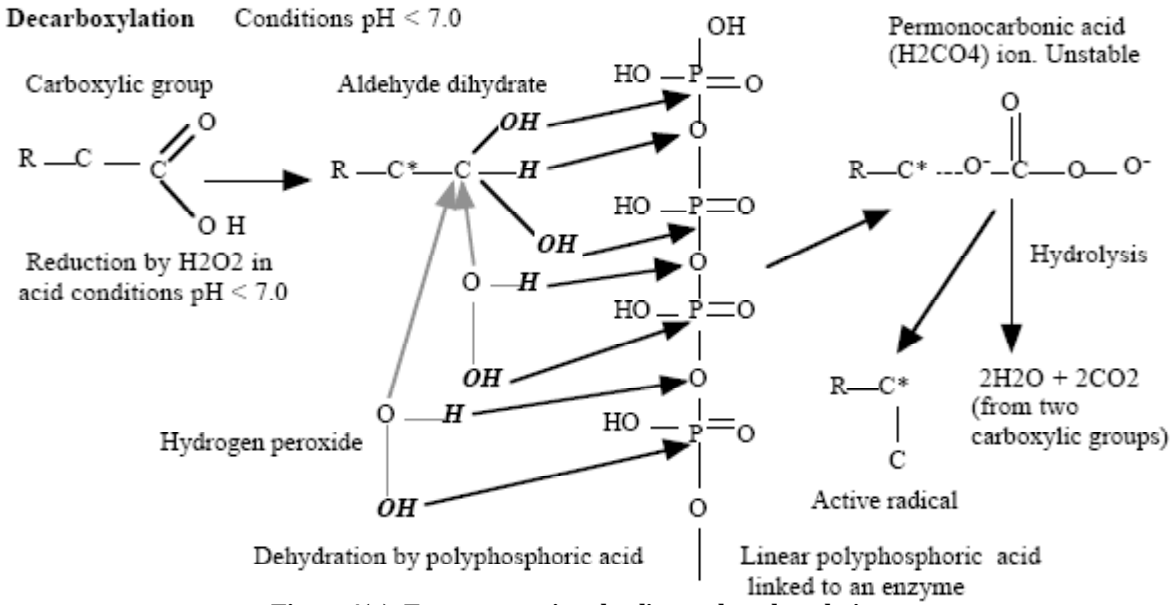

Figure 4(a): Enzyme reactions leading to decarboxylation

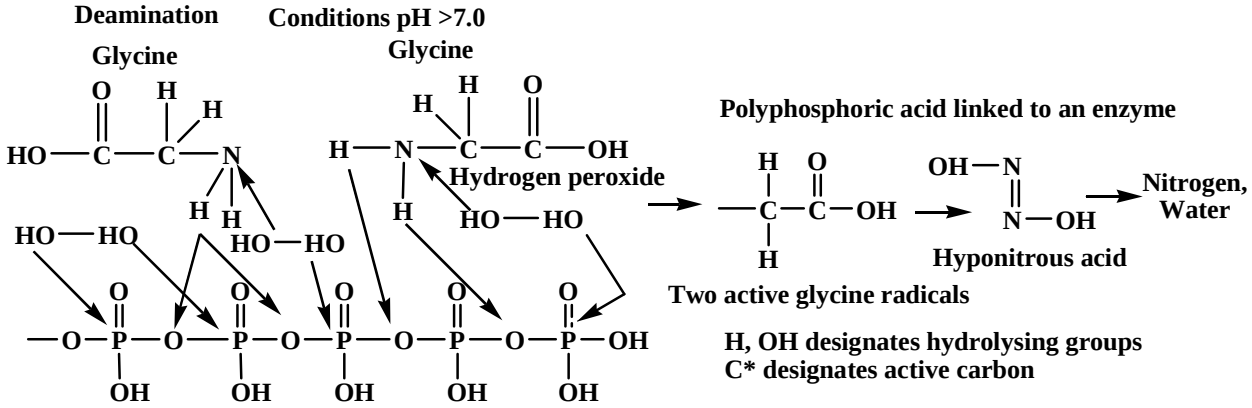

Figure 4(b): Enzyme reactions leading to deamination

strated by the difference in between the plots of reaction of hexokinase and glucokinase with glucose.

Magnesium is the activator of more than 300 enzymes and magnesium-dependant enzymes compose the biggest group[10]. Magnesium ion has been shown to be three times more effective than potassium in catalysing an increased rate of hydrolysis of polyphosphoric acids[4]. This metal ion therefore controls the conversion of enzyme polyphosphoric acid to monophosphoric acid. As is the case for the case of magnesium the presence of other metal ions in an enzyme structure assist or inhibit the conversion of polyphosphoric acid to monophosphoric acid. In addition carbon atoms situated close to carboxyl groups (-CHOH, -CHO, -COOH) are more chemically active than other carbon atoms in the structure. Metal ions in the structure of any enzyme will induce electron displacement in these bonds contributing to enzyme effectiveness.

Specific enzyme supported reactions

The polyphosphate groups associated with an enzyme are also active other biological reactions. The conversion of succinic acid to furmaric acid under the control of an enzyme known as succinate dehydrogenase is shown in figure 3. Oxidising/reducing agents present in biosystems are hydroxylamine and hydrogen peroxide. Hydroxylamine is formed in mammalian cells by the Raschig reaction[11]. The oxidising and reducing characteristics of hydroxylamine and hydrogen perox-

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ide are dependent on the pH of the liquid. When the pH > 7.0 these compounds function as an oxidising agents and when the pH < 7:0 the compounds function as a reducing agents. In the above reaction hydrogen peroxide or hydroxylamine act as reducing agents (pH is < 7.0) giving rise to unstable succinic aldehyde dihydrate. This compound is dehydrated by linear polypho sphoric acid in conjunction with hydrogen peroxide producing fumaric dialdehyde dihydrate which is oxidised by hydrogen peroxide as the pH increases as a result of the formation of monophosphoric acid. The processes of decarboxylation and deamination involving monophosphoric and polyphosphoric acids associated with an enzyme are shown in figure 4a and 4b.

Inhibition of enzymes

Inhibition of enzymes is the observation that the characteristics of particular enzymes can be altered by particular compounds. The effect is not the same as denaturation of enzymes which normally results in the disruption of the enzyme structure. In any given enzyme the effect can be observed as a decrease in the slope of the linear section of the Michaelis-Menton Equation[12] on addition of the inhibitor to the enzyme reaction. Inhibition on the basis of the above is the result of interference with the reversible hydration of polyphosphoric acid. The latter is proposed as being common to all enzymes and it would be expected that any inhibitor observed to affect a given enzyme would affect all enzymes to a greater or less extent. There is no clear evidence that this is the case. However any compound which possess characteristics which can compete with enzymes in enzymic reactions (hydration-dehydration, deamination and decarboxylation etc.) is likely to be an inhibitor. In addition any compound which forms complexes with monophosphoric or polyphosphoric acids will result in the inhibition of enzyme activity. For example peroxymonophosphoric acid (H_3PO_5) and peroxydiphosphoric acid ($H_4P_2O_8$) are formed from monophosphoric acid and hydrogen peroxide. These two acids convert from one to the other in aqueous solution according to the pH of the solution[11]. Hydroxylamine phosphate[$(NH_3OH)_3PO_4$] is formed from monophosphoric acid and hydroxylamine. This complex has reducing characteristics. For example the inhibitor ritonavir contains a considerable number of -NH groups indicating the formation of hydroxylamine phosphate as the inhibitor.

CONCLUSIONS

Enzymes are identified as organic compounds which consist of the combination of a protein associated with active units based on the various spatial forms of polyphosphoric acid or monophosphoric acid. Any protein without the attached polyphosphoric acid molecules will not be chemically active. This is identified with the compound presently called a proenzyme. The effect of the lyotropic series of anions on the characteristics of enzymes has been investigated[14]. Phosphate ion was found to induce the highest stabilisation and the maximum reactivation of a denatured enzyme. This indicates that phosphate is related to enzyme function. Enzyme activity in relation to pH, temperature, enzyme concentration, reactant concentration and spatial orientation of the molecular groups forming organic compounds is mirrored by the stability and activity of polyphosphoric acid with respect to the same factors. The stability of polyphosphoric acid is sensitive to pH value, temperature, nature and concentration of cations present in the fluid. These observations account for the sensitivity of enzymes to these parameters. Metal ions associated with enzyme structure assist the reactions of the latter by controlling the reversible hydration rate of polyphosphoric acid and giving rise to charge displacement which weakens chemical bonds.

The reactions in which enzymes are involved take place between the reactants and monophosphoric acid and polyphosphoric acid for which the protein structure provides support. The transient section of the an enzyme reaction reaction curve represents the establishment of the relative concentrations of mono or polyphosphoric acid according to the conditions in the reaction fluid. This is followed by the linear section of the enzyme reaction rate curve which involves the reaction of the relevant acid and supporting reagents to form giving the observed product. The constant section indicates that an equilibrium state is attained between rate of reformation or the rate of exposure of either mono or polyphosphoric molecules within the protein structure and the rate of the reaction giving rise to the observed products. The specific nature of an enzyme is

decided by the spatial form of the associated polyphosphoric acids (mono, linear, branched linear, hexagonal and quadratic) and the relative molecular concentration of these per unit volume of the enzyme. The specific nature of an enzyme is decided by the spatial form of the associated polyphosphoric acids (mono, linear, branched linear, hexagonal and quadratic) and the relative molecular concentration of these per unit volume of the enzyme.

These proposals are supported by the well known hydrolysing action of pepsinogen on ingested proteins and carbohydrates in the stomach. The observed change in the pH of the of the stomach fluid (chyme) is from a pH value of 2 when the stomach is in the resting condition to a pH value of 6.5 on digestion of a typical meal. On entering the stomach fluid monophosphoric acid distributed throughout the pepsinogen hydrolyses proteins and carbohydrates present in the chyme and is converted to polyphosphoric acid. The reactions of stomach hydrochloric acid with items of the diet produces an increasing pH value in the chyme. As the pH value changes to the optimum value for trypsin this enzyme continues the hydrolysis process at a lower rate and the increasing concentration of polyphosphoric acid initiates dehydration reactions giving rise to other products of the digestive process. Several values of intracellular phosphate ion concentration are recorded in the literature (TABLE 1) and may indicate cellular variations in the concentration of this ion. Under these circumstances the nature of an enzyme formed by a given cell will also vary. Biological chemical reactions involving polyphosphoric acid are examples of stereochemical reactions involving the stereochemistry of an inorganic molecule as well as organic molecules. This, in addition to the fact that metabolic fluids exist a colloids, distinguishes biological chemical reactions from aqueous chemical reactions.

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